of carboxyl groups present, even though they are of high molecular weights. In a similar manner, the celluloses oxidized by nitrogen dioxide dissolve in dilute aqueous solutions of inorganic or organic bases when the carboxyl group content is above about 13%. Products with less than about 13% of carboxyl do not dissolve, probably because there are insufficient carboxyl groups to bring the macromolecules into solution. Additional proof of carboxyl groups is given by the great absorptive capacity for basic dyes and the formation of salts by removal of cations from salts of weaker acids.

As shown by Fig. 5, a typical oxidation curve for the static method, the nitrogen dioxide rapidly oxidizes the cellulose in the early stages of the reaction, and then oxidation practically ceases. The experiments of Table VI, using various nitrogen dioxide/cellulose ratios in containers of various sizes, were run for extended periods of time so that the oxidations would reach completion. When the carboxyl group contents of the oxidized celluloses of Table VI are compared with the nitrogen dioxide/cellulose ratios, the composite graph obtained (Fig. 6) shows a good correlation between the various series.

Though the calcium acetate method of analysis gave results in good agreement with the results of the carbon dioxide evolution method when using celluloses of low degrees of oxidation (Table III), we believe that the latter method is more reliable

at present. Recently, a critical study has been made of the application of the carbon dioxide evolution method to the determination of uronic acids in cellulose materials.[19] A further study of the calcium acetate method might show suitable conditions which would render it satisfactory. This method involves a heterogeneous reaction between a liquid and a solid phase. Complete penetration of the calcium acetate solution into the fibers is necessary for complete reaction with the carboxyl groups. Occlusion or preferential absorption of acetic acid by the fibers would also produce low values.

Summary

1. Methods for oxidizing cellulose by nitrogen dioxide are described.

2. The properties of the oxidized celluloses thus obtained are described.

3. The ability of these oxidized celluloses to dissolve in dilute aqueous alkalies, to absorb large amounts of basic dyes and to replace weaker acids such as acetic acid from their salts indicates extensive carboxyl group formation during oxidation.

4. The analyses of the oxidized celluloses by carbon dioxide evolution by reaction with calcium acetate are reported.

5. The production of salts of the oxidized celluloses by various methods is described.

(19) R. L. Whistler, A. R. Martin and M. Harris, *J. Res. Natl. Bur. Stds.*, **24**, 13 (1940).

Investigation of the Properties of Cellulose Oxidized by Nitrogen Dioxide. I*

BY CORNELIUS C. UNRUH AND WILLIAM O. KENYON

Little is known concerning the structure of oxidized celluloses because various oxidants may simultaneously attack one or more of the hydroxyl groups of the anhydro-glucose units in the cellulose molecule to produce a complicated structure containing carboxyl, aldehyde and ketone groups, or possibly mixtures thereof.[1] Also the products of oxidation are usually heterogeneous and degraded.[2] An exception appears to result when

periodic acid is the oxidant, in which case the secondary hydroxyl groups are preferentially attacked.[3]

The oxidized celluloses prepared by using nitrogen dioxide as the oxidant[4] offer unique possibilities for structural studies. Products covering a wide range of degree of oxidation can thus be prepared, and above a certain degree of oxidation the products are completely soluble in dilute aqueous alkali. Retention of fiber structure and lack of friability of these materials indicate little apparent

* Presented before the Division of Cellulose Chemistry at the St. Louis meeting of the American Chemical Society, on April 10, 1941.

(1) G. F. Davidson, *Silk J.*, **13**, 21–25, No. 145 (1936); *Textile Mfg.*, **62**, 233 (1936); *J. Textile Inst.*, **27**, 144–158 (1936); **29**, 195–218T (1938); *J. Soc. Dyers and Colourists*, **56**, 58–63 (1940).

(2) E. Heuser "Organic Chemistry," H. Gilman, Editor, John Wiley and Sons, New York, N. Y., 1938, Vol. II, pp. 1556–1557.

(3) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 2049 (1937); **60**, 989 (1938).

(4) E. C. Yackel and W. O. Kenyon, *ibid.*, **63**, 121 (1941).

degradation. The present paper describes preliminary investigations of the point of attack of the cellulose molecule by nitrogen dioxide and the nature of the groups formed. Some additional data on the cyclic method of oxidation by nitrogen dioxide and on methods for determining carboxyl groups in oxidized cellulose are included. In this paper the term "nitrogen dioxide" or "NO_2" refers to the equilibrium mixture of nitrogen dioxide and nitrogen tetroxide present under the conditions employed.

Experimental

Preparation of Oxidized Celluloses.—A series of 50-g. batches of oxidized celluloses was prepared from Eastman Filtration Cotton (E. F. C.) by the cyclic oxidation process previously described.[4] The characteristics of the Eastman Filtration Cotton were given in the preceding paper. These were of various degrees of oxidation resulting from variation of the oxidation time. The rate of oxidation by this method is shown in Fig. 1.
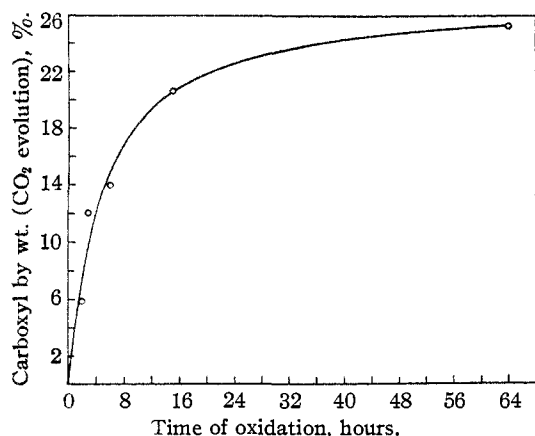

Fig. 1.—Rate of oxidation of cellulose by nitrogen dioxide in the cyclic method.

Analytical Methods.—The oxidized celluloses were analyzed by the calcium acetate and carbon dioxide evolution methods previously described.[4] In addition, two other methods of determining carboxyl group content were used as follows.

In the first of these, designated as the pyridine titration method, the oxidized cellulose was dried overnight *in vacuo* over phosphorus pentoxide and samples of 0.5 to 1.0 g. were weighed into 125-cc. flasks. Thirty cubic centimeters of an aqueous pyridine solution prepared from 1 part by volume of redistilled Eastman Kodak Co. grade pyridine and 2 parts of distilled water were added. The resulting light yellow gelatinous mixture was warmed on the steamplate for fifteen to twenty minutes, which caused the samples containing about 13% or more of carboxyl to dissolve completely. The sides of the flask were washed down with 30 cc. of distilled water, and the solution was titrated with 0.5 N sodium hydroxide solution to phenolphthalein end-point. The values obtained by this method were corrected for a blank titration on the pyridine. These

conditions were chosen from a series of experiments in which other pyridine–water mixtures and various times of heating were employed.

The second method, designated as the alkali–pyridine method is as follows. The samples were dried and weighed as described above. Twenty-five cubic centimeters of 0.5 N aqueous sodium hydroxide was added together with 30 cc. of the 1:1 aqueous pyridine. The samples were shaken for five minutes at room temperature, which caused the more highly oxidized materials to dissolve. The excess alkali was titrated to phenolphthalein end-point with 0.5 N sulfuric acid and the carboxyl group content was calculated from the alkali consumed. In this and the previous method the end-point was indistinct, because of the yellow color of the solutions.

The results obtained with the four methods of analysis are shown in Table I.

TABLE I

COMPARATIVE ANALYSES FOR CARBOXYL CONTENT BY SEVERAL METHODS

Sample	Time of oxidation, hours	% COOH by weight			
		Calcium acetate method	Pyridine titration method	Alkali pyridine method	CO_2 evolution
1	64	22.8	25.8	33.1	25.3
2	15	16.0	17.6	19.7	20.6
3	6	9.2	10.8	12.2	13.9
4	3	6.3	7.1	9.4	12.0
5	2	2.3	3.1	5.7	5.8

Copper Number Determinations.—The copper numbers were determined by the U. S. Forest Products Laboratory modification[5] of the method of Staud and Gray[6] and by the method of Knecht and Thompson[7] for highly oxidized celluloses. The results are given in Table II.

TABLE II

COPPER NUMBERS OF OXIDIZED CELLULOSES

Sample	Forest Products Lab. Method	Knecht and Thompson Method
1	71.0	45.3
2	66.2 (Av. of Table III)	31.0
3	54.2	40.0
4	45.6	36.8
5	28.0	30.3

Difficulty was encountered, especially by the Knecht and Thompson method, in filtering off the cuprous oxide due to residues of highly swollen salts of oxidized cellulose.

When boiled in distilled water, the highly oxidized celluloses gradually dissolved to give nearly colorless solutions which darkened on continued boiling. Evaporation of the aqueous solutions yielded a brown, brittle material indicating profound hydrolysis. The less oxidized materials appeared partially to hydrolyze. In the copper number method, the cellulose was placed in distilled water and heated at 100–101° until the water reached the bath temperature, when the test solutions were added. In our analyses about one-half hour heating was needed to reach

(5) Paper presented by P. B. Sherrard and E. C. Davidson before the Cellulose Division of the A. C. S., University of Wisconsin meeting, May 29, 1926.

(6) C. J. Staud and H. LeB. Gray, *Ind. Eng. Chem.*, 17, 741 (1925).

(7) E. Knecht and L. Thompson, *J. Soc. Dyers and Colourists*, 36, 255 (1920).

equilibrium with the bath. To test the hypothesis that the high copper numbers were due to hydrolysis during this period, portions of sample no. 2 suspended in distilled water were placed in the 101° bath and heated for the times indicated in Table III prior to the copper number determination.

TABLE III
EFFECT OF TIME OF HEATING ON COPPER NUMBER

Time of heating before copper number determination	Copper number
5.5 hours	70.9
0.5 hour	63.4
5 min.	64.4
0	66.1

Esterification.—Ten-gram samples of the oxidized celluloses were allowed to stand for twenty hours at room temperature in a mixture of 72 cc. of Stoddard solvent[8] and 8 cc. of glacial acetic acid. A mixture of 29 cc. of glacial acetic acid, 43 cc. of 95% acetic anhydride, 38 cc. of Stoddard solvent, 8 cc. of trichloroethylene, and 0.1 cc. of concentrated sulfuric acid was added to each sample. After thorough mixing, acetylation was allowed to proceed at 40°, for ninety-two hours with periodic agitation. The fibrous esters were separated from the esterification mixtures, washed with alcohol, then with distilled water until the washings were neutral, and air-dried.

The esters, dried in vacuo over phosphorus pentoxide, were analyzed for apparent acetyl contents by the modified Knoevenagel method[9] and by a distillation method (Table IV).[10] Since sample no. 1 darkened during acetylation, its analyses are not included.

TABLE IV
ACETYL CONTENTS OF ACETYLATED OXIDIZED CELLULOSES

Sample	Apparent acetyl, % Knoevenagel method	Actual acetyl % distillation method	Actual acetyl, % calculated
2	59.3	33.0	35.2
3	56.0	38.4	38.9
4	52.5	38.4	39.8
5	48.7	41.4	42.5
Check fibrous acetate from E. F. C.	44.3	42.4	44.8
Check hydrolyzed acetate	40.6	39.3	..

In the modified Knoevenagel method, the acetates are hydrolyzed by heating with sodium hydroxide solution and the excess alkali is titrated. The apparent acetyl content thus calculated from alkali consumption is high because of the alkali neutralized by the carboxyl groups of the oxidized celluloses. It was reported in a previous paper[4] that heating of oxidized cellulose with alkali gives very high apparent carboxyl group contents, and this behavior may also be partly responsible for the high apparent acetyls. The distillation method consists of an initial alkaline hydrolysis followed by addition of phosphoric acid and vacuum distillation of the volatile acid. The acid in the dis-

tillate is determined and calculated as acetic acid. This method of determining combined acetic acid is not complicated by the carboxyl groups.

Furfuraldehyde Determinations.—A standard method using the usual "pentosan apparatus"[11] was employed for evolving furfuraldehyde which was determined as the 2,4-dinitrophenylhydrazone.[12,13] The identity of the hydrazone was checked by melting point and nitrogen analysis (found 20.92% N, calculated 20.29). The results obtained are shown in Table V and compared with similar determinations on alginic acid and pectic acid which contain combined mannonuronic acid and galacturonic units, respectively. Pectic acid also contains pentosans, consequently it gave a somewhat higher furfuraldehyde value.

TABLE V
FORMATION OF FURFURALDEHYDE FROM OXIDIZED CELLULOSES

Sample	% COOH (by wt.) CO₂ evolution method	Furfuraldehyde formed (% by wt.)
Oxidized cellulose no. 6	20.5	8.8
Oxidized cellulose no. 7	22.9	9.5
Oxidized cellulose no. 8	22.8	9.8
Alginic acid		16.6
Pectic acid	22.1	20.9

Discussion

From Table I it will be seen that in the cyclic oxidation method, the degree of oxidation is a function of time. If the time is prolonged, the carboxyl group contents of the oxidized materials reach a limiting value of about 25% (Fig. 1). The calculated carboxyl group content of a poly-anhydroglucuronic acid is 25.5%. A comparison of the analyses by various methods (Table I) indicates that the calcium acetate method and the pyridine titration procedure gave low results as compared with the results of the carbon dioxide evolution method. The lack of agreement between the results of the calcium acetate and carbon dioxide evolution method was unexpected since a comparison of these analytical methods on materials produced by the static oxidation method gave better agreement.[4] The values of the alkali-pyridine and the carbon dioxide evolution methods are reasonably close except for sample no. 1, where a very high value was obtained in the presence of alkali. In the previous paper of this series[4] the sensitivity of these oxidized celluloses to alkali has been mentioned. We believe that the analyses by carbon dioxide evolution are more

(8) A commercial hydrocarbon fraction boiling between 150 and 200°.

(9) T. F. Murray, Jr., C. J. Staud and H. LeB. Gray, Ind. Eng. Chem., Anal. Ed., 3, 269 (1931).

(10) We wish to thank Dr. L. B. Genung for these analyses.

(11) "Methods Used at the Forest Products Laboratory for the Chemical Analysis of Pulps and Pulpwoods," compiled by M. W. Bray, revised September, 1939, pp. 32–37. See also Bray, Paper Trade J., 87, 59 (1938).

(12) H. Reynolds, O. L. Osborn and C. H. Werkman, Iowa State College, J. Science, 7, 443 (1933).

(13) H. A. Iddles and C. E. Jackson, Ind. Eng. Chem., Anal. Ed., 6, 454 (1934).

accurate than those obtained by the three other methods, since they are presumably not complicated by side reactions due to alkali or by incomplete penetration of the fibers by solutions. Hence, we use the values obtained by carbon dioxide evolution in describing our products.
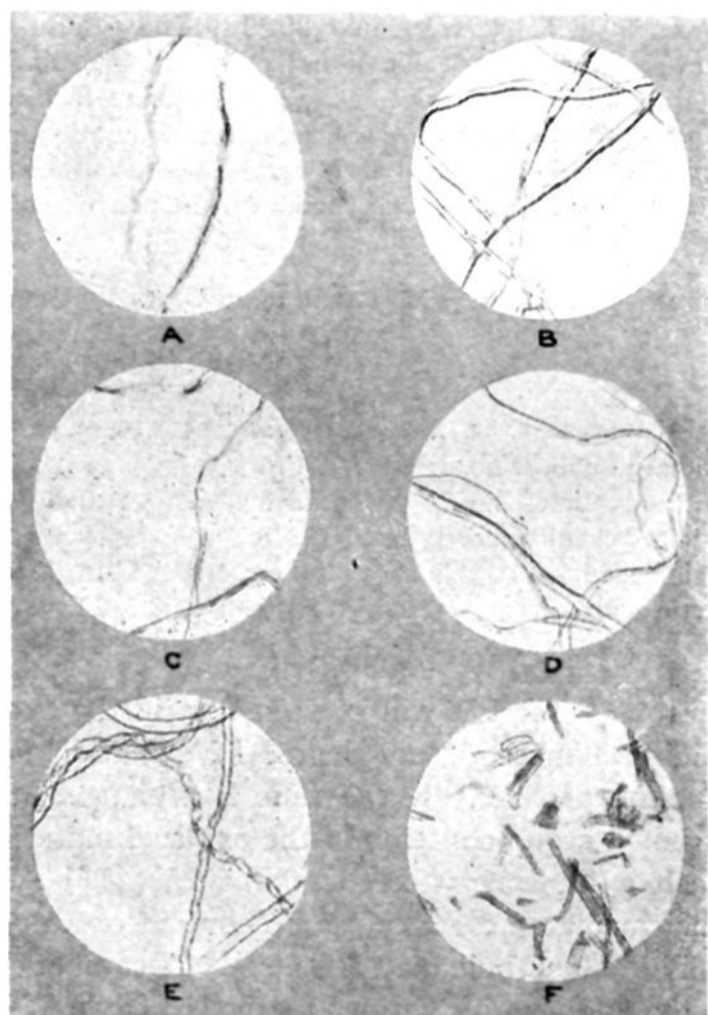

Fig. 2.—Magnification = 100×: A, Eastman Filtration Cotton; B, E. F. C. oxidized by NO_2, COOH = 5.8%; C, E. F. C. oxidized by NO_2, COOH = 13.9%; D, E. F. C. oxidized by NO_2, COOH = 20.6%; E, E. F. C. oxidized by NO_2, COOH = 24.0%; F, Eastman Filtration Cotton oxidized by $Mg(MnO_4)_2$ according to a standard method.

The very high values for copper numbers obtained by the Forest Products Laboratory method are very interesting. The value of 71.0 for sample no. 1 is in good agreement with the theoretical copper number of 72.2 for anhydroglucuronic acid calculated on the basis of the reducing power of one aldehyde group for each uronic acid unit. The value of 28.0 for the copper number of sample 5 is about twice the calculated copper number for this sample on the basis of uronic acid content calculated from carbon dioxide evolution. The copper numbers of samples 2, 3, and 4 are somewhat higher than the values of 58.0, 39.7 and 33.7 calculated from uronic acid contents on the basis of one aldehyde for each uronic acid unit.

It has been previously assumed that the copper numbers measured free reducing groups in the oxidized cellulose arising either from hydrolysis of the hemiacetal linkages or from oxidation of a hydroxyl group to aldehyde. These high values would necessitate extensive degradation with concomitant loss of fiber structure if they are due to aldehydes arising from hydrolysis of oxygen bridges between anhydroglucose units during the oxidation process. We have repeatedly pointed out in this and in a previous paper that the fibrous structure is maintained during the oxidation with nitrogen dioxide. This is shown in Fig. 2, in which photomicrographs of the original cellulose, of four samples of nitrogen dioxide oxidized celluloses and a cellulose oxidized by a permanganate are compared. These results would lead to the belief that the reducing groups being measured by the copper number are not present as such in the free state in the oxidized cellulose but arise by fission during heating in the alkaline copper solutions. The dependence of the copper number upon the degree of oxidation suggests that the uronic acid units may hydrolyze with ease, thus producing the aldehyde groups which are determined by the copper number test. Davison[14] has pointed out that other types of oxidized cellulose show, likewise, alkali sensitivity. As previously shown, the more highly oxidized samples hydrolyzed on boiling with distilled water to yield water-soluble hydrolysis products.

The results of acetylation experiments (Table IV) show that acetates of high acyl contents may be produced from oxidized celluloses. The determination of acyl contents of such esters by the modified Knoevenagel method is unreliable for the reasons pointed out in the experimental part of this paper though this analytical method is quite satisfactory for cellulose acetates of unoxidized cellulose. These results are included only to show the behavior of acetates of oxidized celluloses under such conditions. In the present case, acetyl determinations by distillation are much more reliable and the values thus obtained agree reasonably well with the calculated values (Table IV). These theoretical values were calculated on the following basis. From the carbon dioxide evolution determinations, the percentage of combined uronic acid was calculated and the remainder was assumed to be unattacked anhydroglucose units. From the percentage of anhydro-

(14) G. F. Davison, J. Soc. Dyers and Colourists, 56, 58 (1940).

glucose units, which were considered to yield a triacetyl derivative, and the percentage of combined anhydrouronic acid groups, which were considered to yield a diacetate, the theoretical acetyl values for the acetates of oxidized celluloses could be derived.

The literature contains many determinations of the production of furfuraldehyde from various oxidized celluloses, but the yields are usually low.[15] As will be seen in Table V, the celluloses oxidized by nitrogen dioxide yield furfuraldehyde to the extent of 9 to 10% by weight. The theoretical yield of furfuraldehyde from a polyanhydrouronic acid is 54.6%, but it has been shown that glucurone itself on distillation with 12% hydrochloric acid yields only about one-third of a theoretical amount of furfuraldehyde.[16] On this basis, and considering the amount of combined uronic acid indicated by carbon dioxide evolution, our yields of furfuraldehyde are about 60% of that which might be expected, but it does indicate a very high content of combined uronic acid groups. The yield of furfuraldehyde from alginic acid and pectic acid is about what one would expect from the results on glucurone.

The fact that the carbon dioxide evolution values agree reasonably well with the carboxyl group determinations by other methods would indicate that there are no substantial amounts of carboxyl groups present other than those in the combined uronic acid units. If carboxyl groups were formed by oxidation at other points in the anhydroglucose unit, the carboxyl group contents determined by the calcium acetate or titrimetric methods would be expected to be higher than those determined by carbon dioxide evolution, and such is not the case. The high furfuraldehyde contents corroborate this view of the presence of large amounts of combined uronic acid units. If, in addition to the hydroxyls oxidized to carboxyls, other hydroxyl groups in the molecule are oxidized, we would expect a considerable difference between the calculated and actual acetyl contents of the esters. However, the acetylation data seem to account for the hydroxyl groups which are not oxidized to carboxyl. On the basis of these considerations, the evidence points to the fact that oxidation by nitrogen dioxide preferentially attacks the primary hydroxyl groups of the cellulose and would lead to the belief that the

partially oxidized materials produced by this method are made up of chains of anhydroglucose and anhydroglucuronic acid units. The carboxyl group content of 25.3% which was found for the highly oxidized cellulose (Sample 1), agrees well with the calculated value for a material composed only of anhydroglucuronic acid units. This apparent selective attack of the primary hydroxyl groups of cellulose by nitrogen dioxide is an interesting contrast to the action of periodic acid which attacked preferentially the secondary hydroxyl groups.[3] In the presence of an excess of oxidant, the production of carboxyl groups practically ceased when the carboxyl group content approached the theoretical value for polyanhydroglucuronic acid. This is additional evidence of the selective oxidation of one hydroxyl group of each anhydroglucose unit to a carboxyl group.

In order to test these results further, work is in progress on the identification of hydrolysis and acetylosis products. This approach is complicated by the fact that uronic acids under hydrolytic conditions usually break down into other compounds such as furfuraldehyde. However, acetylosis of a partially oxidized cellulose has yielded some cellobiose octaacetate, which is in agreement with the hypothesis that the partially oxidized materials may consist in part of unoxidized anhydro-glucose units.

Summary

1. The cyclic method of oxidizing cellulose by nitrogen dioxide has been investigated further. The products appear to approach about 25% of carboxyl group content as a limiting value.

2. The oxidized celluloses have high apparent copper numbers. It is suggested that these arise from reducing groups formed during the determination rather than from free reducing groups already present.

3. The highly oxidized celluloses yield furfuraldehyde to the extent of 9 to 10% by weight. The analyses for carboxyl groups indicate that all the carboxyls present appear as uronic acids in the carbon dioxide evolution test. Acetylation of residual hydroxyl groups accounts for those which are not oxidized to carboxyl.

4. The results of (3) would indicate that the nitrogen dioxide preferentially attacks the primary hydroxyl groups of the cellulose to yield oxidized materials containing combined uronic acid units.

(15) E. Heuser and F. Stöckigt, *Cellulosechemie*, **3**, 61 (1922).
(16) K. U. Lefèvre and B. Tollens, *Ber.*, **40**, 4513 (1907).